ISSN: 2574-1241
DOI: 10.26717/BJSTR.2018.11.002121
Merike Vaher. Biomed J Sci & Tech Res

BIOMEDICAL Journal of Scientific & Technical Research

Short Communication Open Access

# Extraction and Analysis of Bioactive Compounds from *Dipsacus Fullonum* and *Galium Verum* for Lyme Borreliosis Treatment

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**Received:** November 22, 2018; **Published:** November 29, 2018

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**Abstract**

Four different methods for the extraction of bioactive compounds from the two medicinal plants were compared. Based on qualitative analysis and comparison of chromatograms, all extracts can be characterized by a high content of polyphenols and iridoids. The identification of major bioactive compounds in both plant extracts were carried out using HPLC-MS analysis. More than fifteen different constituents were identified. The highest amount of iridoid glucosides were observed in Dipsacus samples.

**Keywords:** Bioactive Compounds, Dipsacus Fullonum, Galium Verum, HPLC-MS, Extraction, Lyme Borreliosis

**Abbreviations:** HWE - Hot Water Extraction; PHWE - Pressurized Hot Water Extraction

## Introduction

Lyme borreliosis, although new, is the most common zoonotic disease in Europe, with an estimated 650 000 – 850 000 cases total and a higher incidence rate in Central Europe [1]. Treatment with antibiotics is not always effective [2] therefore, it seems logical to turn to medicinal plants for new treatment options. Liebold et al. showed that lipophilic extract from *Dipsacus sylvesteris* has activity against Borrelia burgdorferi [3]. *Dipsacus fullonum* has been used as a case study to show a promising herb that simply has no research on whether it helps people with Lyme borreliosis or any tick-borne infection [4]. Several herbal lymphatics, including *Galium verum*, can also be used for treatment [5]. Iridoids and polyphenols are present in many medicinal plants and their strong anti-inflammatory effects have been proven [6].

Extraction is the crucial first step in the analysis of medicinal plants. It is necessary to extract these desired chemical components from plants for further separation and characterization. For extraction, different methods were used. Mazina et al. successfully used 80% methanol and ultrasonic bath for the extraction of bioactive compounds from medicinal plants [7]. The aim of the study was to compare the different extraction methods, determine the most suitable for the extraction of bioactive compounds, and identify the major constituents.

## Materials and Methods

**Extraction Procedure**: 1 g of dried plant was treated with 10 ml of 80% (v/v) methanol or 70% (v/v) ethanol and sonicated for 30 min at 40 °C. For Hot Water Extraction (HWE) – 10 mL of boiling water was added to 1 g of plant material and sonicated for 30 min at 80 °C. Pressurized Hot Water Extraction (PHWE) was carried out using 100 mL autoclave, produced by NWA analytics Meßgeräte GmbH. 6 g of dried material and 60 mL of water were loaded into the reactor. Extraction was carried out for 30 min at 80 bar and 100 °C. All obtained extracts were centrifuged for 15 min at 4000 rpm and the supernatant was diluted in accordance with needs.

## Results and Discussion

The efficiency of the extraction methods was evaluated by quantitative analysis of four phenolic compounds – chlorogenic acid, rutin, protocatechuic acid and caffeic acid. The obtained results did not clearly favor one method. HWE and PHWE provided the most effective extraction of chlorogenic acid (6.9± 0.3 mg/g and 6.7±0.3 mg/g respectively) from *Galium verum*. At the same time, the highest yield of this acid in *Dipsacus fullonum* was obtained with methanolic and ethanolic extractions (8.2±0.3 mg/g and 8.1±0.3

mg/g respectively). Protocatechuic and caffeic acids were detected only in *Dipsacus* samples and there are no notable differences in extraction yields between the extraction methods (except PHWE). Rutin was found only in *Galium* samples and the highest content of this compound was obtained with PHWE (4.0±0.1 mg/g) and methanol extraction (3.6±0.1 mg/g).

HPLC-DAD-MS was used for the characterization of the phenolic profiles in different extracts of *Dipsacus fullonum* and *Galium verum*. The identification of the compounds was done by comparison of obtained mass spectra, retention time as well as the UV spectra with standards. In the absence of standards, identification of the compounds was carried out by careful interpretation of MS-MS data and exact measurement of precursor and fragment ions. As the chromatographic analysis shows, all extracts of the different species had a high phenolic content. Six major phenolic compounds were present in the *Dipsacus* samples (predominantly flavone glucosides). Additionally, a significant amount of iridoids were also detected. In *Galium* samples, fourteen major compounds, including quinic acid derivatives, flavonol glucosides, and iridoid glucosides, were identified. Some of the compounds were identified for the first time. The full list of identified compounds is given in Table 1.

**Table 1:** Identification of compound by HPLC-DAD-MS.

| Peak | $R_t$ (Min) | $\lambda_{max}$ | Molecular Ion(M-H)- (M/Z) | MS-MS Data (M/Z) | Tentative Identification |
|---|---|---|---|---|---|
| ***Dipsacus fullonum*** | | | | | |
| 1 | 7.9 | 326 | 353 | 191 | Chlorogenic acid |
| 2 | 9.6 | 340 | 593 | 473,431,312 | Apigenin-di-glucoside |
| 3 | 9.9 | 340 | 447 | 429,358 | Luteolin-hexoside |
| 4 | 10.9 | 340 | 431 | 341,312 | Apigenin-hexoside |
| 5 | 12.2 | 330 | 515 | 353 | Dicaffeoylquinic acid isomer |
| 6 | 12.7 | 330 | 515 | 353 | Dicaffeoylquinic acid isomer |
| 7 | 13.0 | 225 | 585 | 553,375 | Iridiod |
| 8 | 13.9 | 232 | 583 | 551,373 | Sylvestroside |
| ***Galium verum*** | | | | | |
| 1 | 4.3 | 215 | 389 | 345 | Deacetyl-asperulosidic acid isomer |
| 2 | 4.9 | 215 | 389 | 345 | Deacetyl-asperulosidic acid isomer |
| 3 | 6.0 | 218 | 431 | 251 | asperulosidic acid |
| 4 | 6.7 | 326 | 353 | 191 | Neochlorogenic acid |
| 5 | 7.3 | 227 | 431 | 371,191 | Epi-acetylscandoside |
| 6 | 7.9 | 326 | 353 | 191 | Chlorogenic acid |
| 7 | 8.3 | 237 | 459 | 413 | Asperuloside glucoside |
| 8 | 8.8 | 326 | 353 | 191 | Cryptochlorogenic acid |
| 9 | 10.8 | 354 | 609 | 301 | Querceitin-rutinoside (Rutin) |
| 10 | 11.3 | 350 | 447 | 285 | Kaempferol-glucopyranoside |
| 11 | 11.9 | 354 | 623 | 315 | Isorhamnetin- rutinoside |
| 12 | 12.2 | 330 | 515 | 353 | Dicoffeoylquinic acid isomer |
| 13 | 12.5 | 223 | 519 | 357,191 | Iridoid glucoside |
| 14 | 12.7 | 330 | 515 | 353 | Dicoffeoylquinic acid isomer |

## Conclusion

HPLC-DAD-MS is a powerful and accurate method for the separation, identification and quantification of bioactive compounds in different plants. All extraction methods are suitable for the efficient extraction of phenolic and iridoic compounds from different plant matrices. Considering that pressurized hot water and hot water extractions belong to environmentally friendly/ benign extraction methods, this type of sample treatment could be preferable.

## Acknowledgment

Estonian Research Council (Institutional Research Fund No. 33-20) is acknowledged for financial support.

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**ISSN: 2574-1241**

*DOI: 10.26717/BJSTR.2018.11.002121*

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